

## Capillary zone electrophoresis separations of enantiomers present in complex ionic matrices with on-line isotachophoretic sample pretreatment

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### Abstract

Analytical capabilities of capillary zone electrophoresis (CZE) with on-line coupled capillary isotachophoresis (ITP) sample pretreatment in the column-coupling capillary electrophoresis equipment to separate and determine enantiomers present in multicomponent ionic matrices were studied. Tryptophan was used as a model analyte in the ITP-capillary zone electrophoresis experiments performed in this context while a 90-component model mixture of UV-light absorbing organic anions and urine served as multicomponent sample matrices. Various working modes in which the on-line coupled capillary isotachophoresis–capillary zone electrophoresis combination in the column-coupling separation system can operate were employed in the anionic regime of the separation with direct injections of the samples. Advantages and limitations of these working modes in the separations of enantiomers present in model and urine matrices were assessed. Experiments with model mixtures of tryptophan enantiomers revealed that the two were resolved in the capillary zone electrophoresis stage with the aid of  $\alpha$ -cyclodextrin also when their concentration ratio in the sample was 1:200 while the concentration of L(–)-tryptophan was 25 nmol/l. The limits of detection for the enantiomers were at  $\sim 10$  nmol/l ( $\sim 1.5$  ng/ml) concentrations for a 220 nm detection wavelength of the UV detector employed in the capillary zone electrophoresis stage and for a 30  $\mu$ l sample load. A high sample load capacity of the on-line coupled capillary isotachophoresis stage was effective in separating the samples corresponding to 3–6  $\mu$ l volumes of undiluted urine. The results from the runs with urine samples showed that only the capillary isotachophoresis–capillary zone electrophoresis combination with a post-column on-line coupled capillary isotachophoresis sample clean-up (responsible for a removal of more than 99% of the sample anionic constituents migrating in the on-line coupled capillary isotachophoresis stack and detectable in the capillary zone electrophoresis stage) provided a universal alternative for the detection and quantitation of the model analyte (L(–)-tryptophan). © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Enantiomer separation; Isotachophoresis; Sample handling; Tryptophan; Amino acids

### 1. Introduction

Recent reviews and monographs show ([1–5]) that capillary zone electrophoresis (CZE) and micel-

lar electrokinetic chromatography (MEKC) are gaining key positions in the analytical separations of enantiomers. Both CZE and MEKC already proved their practical applicabilities, especially, in the analysis of enantiomers of pharmaceutically important compounds [4,5]. However, the use of these capillary electrophoresis (CE) techniques to the analysis of

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enantiomers present in samples of biological origins is still very rare (see, e.g., refs. [5–8]). The following facts summarize probably main hindrances preventing a wider use of CZE and MEKC in this important application area:

1. Samples of biological origin (e.g., serum, plasma, urine) usually contain inorganic constituents at very high concentrations. This is limiting the sample volumes that can be loaded onto the column as otherwise an undesired sample stacking occurs [9].
2. Some types of biological samples (e.g., serum, plasma) contain proteins at (very) high concentrations. A negative role of proteinaceous matrix in the CE separations of enantiomers may be manifold [7].
3. Very high separation efficiencies as attainable in CZE and MEKC cannot eliminate risks of the analyte peak overlap linked with multicomponent natures of some biological matrices (e.g., urine). This is reflecting common limits of the column separation techniques as discussed by Giddings [10].
4. Low concentration limits of detection (often essential in the determination of enantiomers present in biological samples) cannot be achieved in current, low sample load capacity CZE and MEKC columns unless extremely sensitive detection techniques are used.

One way to solve the above problems is the use of very sensitive and selective detectors. A direct CZE determination of zolpidem and its main metabolites in urine with the aid of laser-induced fluorescence detection may serve as an illustration of the use of such an approach [11]. In general, however, the above facts clearly indicate that the use of appropriate sample preparation techniques may be essential in the CZE and MEKC determinations of enantiomers present in biological matrices. Sample preparation techniques based on various separation principles have been combined with the CZE and MEKC separations (e.g., liquid–liquid extraction [7,12,13], solid-phase extraction [14–16], dialysis [17,18], electrodialysis [19], ultrafiltration [7] and electroseparation techniques [20–30]) and these offer sample pretreatment alternatives suitable to the separations of enantiomers present in the samples of biological origin.

To our best knowledge there is only one work in which the column-coupling CE separation system was used to the CZE separations of enantiomers [31]. In this work the carrier electrolytes with and without chiral selectors were used in the coupled columns to improve the detection conditions for the enantiomers. In this context we should note that the column-coupling CE separation system is analytically very beneficial when it on-line couples CZE with capillary isotachopheresis (ITP) sample pretreatment. This ITP–CZE combination, integrating the separation and sample pretreatment steps into one electrophoretic run, was already proved to be very effective CE technique in (ultra)trace analysis of ionogenic compounds present in complex (ionic) matrices [20–30]. So far, however, no attention was paid to its use to the separations of enantiomers present in biological fluids.

The aim of the present work was to study analytical capabilities of the ITP–CZE combination to separate and determine enantiomers present in multicomponent ionic matrices and in matrices containing, in addition, inorganic electrolytes at very high concentrations. Tryptophan was chosen as a model analyte for our experiments while a 90-component model mixture of UV-light absorbing organic anions and urine served as multicomponent sample matrices. Various working modes in which the ITP–CZE combination in the column-coupling separation system can operate were employed in the anionic regime of the separation with direct injections of the samples. Advantages and limitations of these working modes in the separations of enantiomers present in complex biological matrices were assessed.

## 2. Experimental

### 2.1. Instrumentation

A laboratory assembled column-coupling capillary electrophoresis instrument [32,33] was used in the ITP–CZE separations carried out in this work. Its separation unit consisted of the following subunits (see a schematic drawing in Fig. 1): (i) An ITP valve injector (a 30  $\mu$ l internal sample loop); (ii) an ITP pre-separation column [provided with a 800  $\mu$ m I.D.

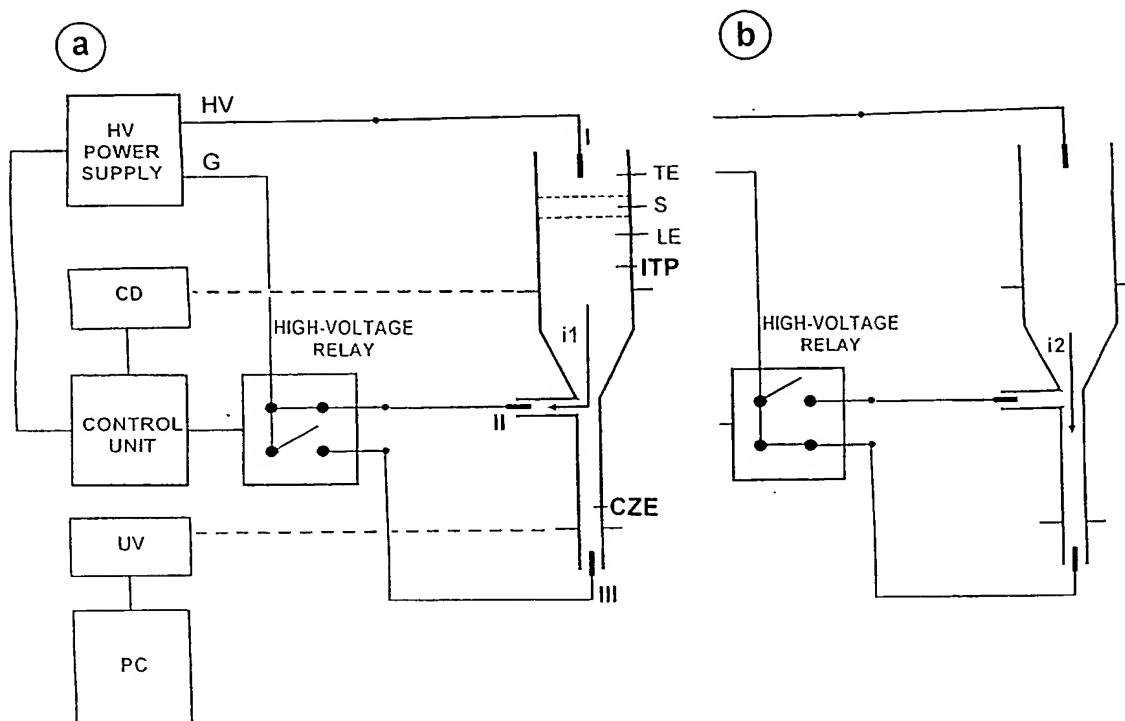


Fig. 1. A block scheme of the column-coupling capillary electrophoresis equipment as used in this work and its running modes. a=a path of the driving current effective during the separation in the ITP column and in the post-column ITP sample clean-up; b=a path of the driving current effective during the transfer of the analyte containing sample fraction onto the CZE column and in the final separation of the sample fraction in the CZE column. The terminating electrode (I) of the separation unit was permanently connected to the high-voltage (HV) pole of the power supply. The counter-electrodes of the ITP and CZE columns (II and III, respectively) were permanently connected to a high-voltage relay. Moving elements of this switching device connected the ground pole (G) of the power supply either to the counter-electrode of the ITP column (a) or to the counter-electrode of the CZE column (b). In this way the direction of the driving current (transport of the sample constituents) was determined (arrows  $i_1$  and  $i_2$ ). The sample (S) was placed by a valve (a 30  $\mu$ l internal sample loop) between the leading (LE) and terminating (TE) electrolytes before the separation [see (a)]. CD=conductivity detector for the ITP column (used to monitor the ITP separation and control the switching of the columns via the control unit); UV=UV photometric absorbance detector (used to monitor the separation in the CZE stage). Control unit=a controller for an unattended operation of the equipment during the electrophoretic run.

capillary tube made of FEP (fluorinated ethylene-propylene copolymer) with an on-column conductivity sensor] of a 90 mm length; (iii) a bifurcation block for an on-line coupling of the ITP and CZE columns; (iv) counter-electrode compartments for both the ITP and CZE columns. These subunits were bought from Villa-Labeco (Spišská Nová Ves, Slovakia). A 300  $\mu$ m I.D. capillary tube made of FEP (Villa-Labeco) was used for the CZE separations in the second stage of the ITP–CZE combination.

A Spectra Focus multiwavelength photometric

absorbance detector (Thermo Separation Products, S.Jose, CA, USA) was used for the detection in the CZE stage. A CZE 1000 high voltage power supply (Spelman, Hauppauge, NY, USA) delivered the stabilized driving current. A laboratory constructed controller of the instrument included also electronics for the column-switching relay and for the conductivity detector of the ITP column.

The data from the photometric detector in the CZE stage were acquired and processed by a PC 1000 Candidate (version 3.0) program from Thermo Separation Products.

## 2.2. Chemicals and the samples

Chemicals used for the preparations of the electrolyte solutions and model samples were obtained from Fluka (Buchs, Switzerland), Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Lachema (Brno, Czech Republic) and Merck (Darmstadt, Germany). Methylhydroxyethylcellulose 30 000 (MHEC) obtained from Servaserved as a suppressor of the electroosmotic flow in the separation compartment [34]. The electrolyte solutions were prepared in demineralized water. The electrolyte solutions were filtered through disposable membrane filters (a 1.2  $\mu\text{m}$  pore size) before the use.

A 90-component model mixture consisting of aromatic carboxylic and sulfonic acids, nucleotides and amino acids was prepared from chemicals provided by the above suppliers. An aqueous stock solution of the model mixture containing these anionic constituents at 1–5 mmol/l concentrations was kept at  $-20^{\circ}\text{C}$  in a refrigerator. A fresh solution of the model mixture taken for the CE experiments was prepared from this stock solution by its dilution with demineralized water.

## 3. Results and discussion

In the ITP–CZE combination as used in this work we divided the analysis into two well defined stages. In the first stage we performed the ITP concentration of tryptophan and, at least, an in-column sample clean-up (a removal of the sample constituents which cannot reach the outlet of the ITP column under actual ITP working conditions [35]). CZE performed in the second stage served for a resolution of tryptophan enantiomers and a final separation of the sample constituents transferred into this stage with the amino acid. The composition of the electrolyte system used in our experiments (Table 1) reflected these analytical functions of both separation stages.

The column-coupling CE separation system [32,33] offers several working modes for the ITP–CZE combination [20]. The ones employed in this work included:

1. CZE combined with a transient ITP sample stacking (A, in Fig. 2). This working mode was in fact equivalent to transient ITP as used in the CZE separations in single column CZE instruments [9]. In the ITP stage of this mode (see, A1, in Fig. 2) the sample constituents were concentrated and (partially) separated by ITP into a series of contiguous ITP zones. A complete ITP stack was then transferred into the CZE stage for a final separation (A2, in Fig. 2).
2. CZE combined with a transient ITP sample stacking with the analyte spaced from the rest of the sample constituents by a pair of discrete spacers (B, in Fig. 2). This working mode had an identical working scheme as the first one. The only difference was in the use of the spacing constituents in the sample (the sample constituents 1 and 2 in Fig. 2 (B)) to spatially separate tryptophan from the sample constituents stacked in its neighbourhood in the ITP stage [20,36]. In this way tryptophan was injected into the CZE stage for a final separation as a defined analyte pulse in the ITP stack of the sample constituents (see, B2, in Fig. 2).
3. CZE combined with a post-column ITP sample clean-up (C, in Fig. 2). This working mode introduced a post-column clean-up of the analyte into the ITP–CZE combination. This clean-up operation was performed at the outlet of the ITP column (see Fig. 2 (C)). Here, the spacing constituents determined the fraction in which tryptophan was transferred into the CZE stage for a final separation. The sample constituents migrating in front of the first and behind the second spacers (see, C1, in Fig. 2) were removed from the separation compartment in the way shown in Fig. 2 (C) by appropriately switching the high voltage relay (Fig. 1).

### 3.1. Separating conditions and limits of detection for tryptophan enantiomers

The leading and terminating electrolytes employed in the first separation stage (see Table 1) favoured the ITP resolution of tryptophan from the rest of the sample constituents. In preliminary experiments we found that  $\alpha$ -cyclodextrin resolves the tryptophan enantiomers in the anionic CZE separations under the electrolyte conditions described in Table 1 and no other chiral selectors were tested in this respect. The terminating anion of the electrolyte system

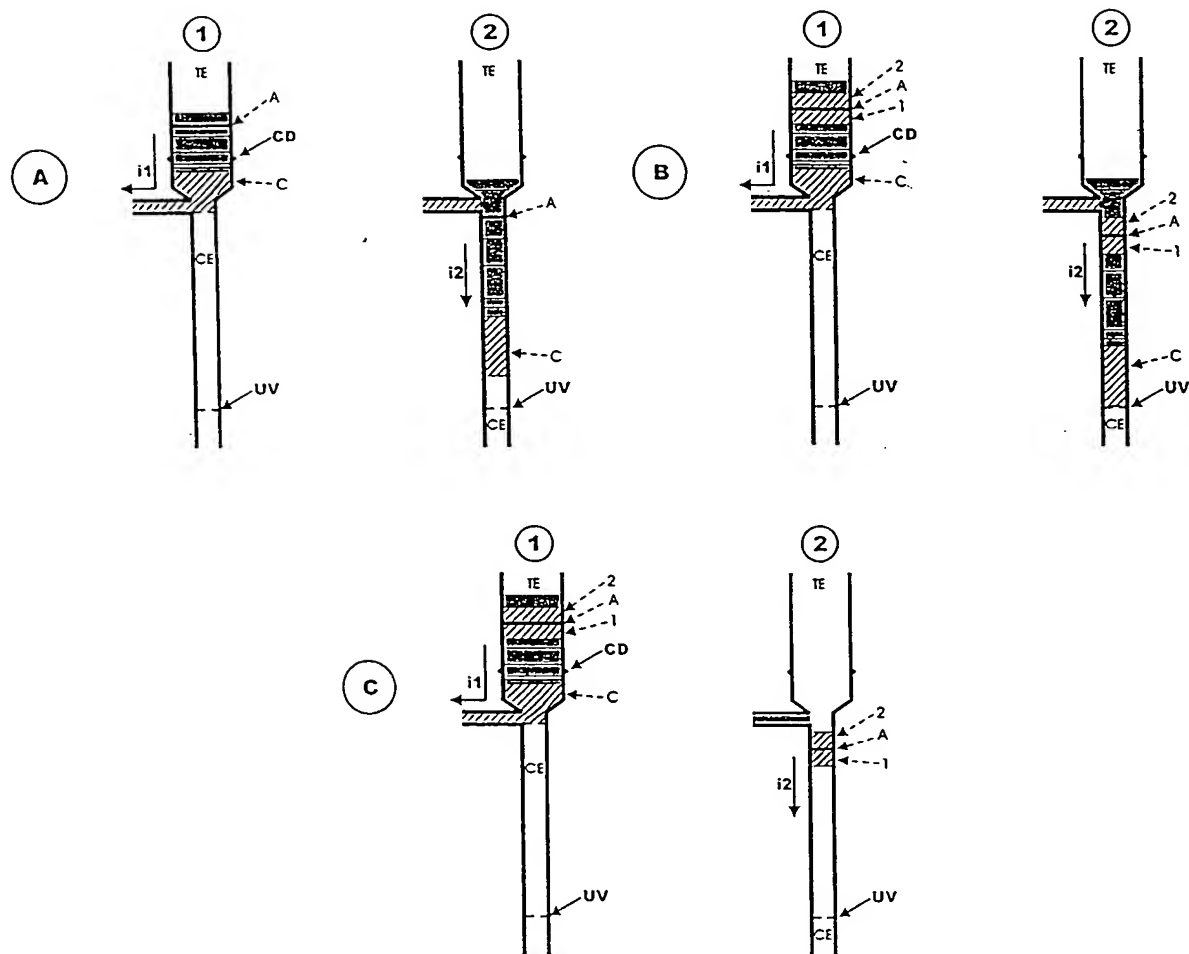


Fig. 2. Working modes of the ITP-CZE combination in the column-coupling capillary electrophoresis equipment as used in this work. (A)=CZE combined with a transient ITP sample stacking; (B)=the same as in (A) only the sample contained a pair of discrete spacers (1,2) for a spatial separation of the analyte from the matrix constituents; (C)=CZE combined with a post-column ITP sample clean-up. Characteristic situations in the ITP and CZE stages before the final CZE separations: A1,B1=final situations after the separations in the (first) ITP stage of the ITP-CZE runs; C1=a situation in the ITP stage before a post-column ITP clean-up [this included electromigration removals of the sample constituents present in the ITP stack and migrating in front of the front spacer (1, in C1 and C2) and behind the rear spacer (2, in C1 and C2)]; A2-C2=starting situations in the (second) CZE stage of the ITP-CZE runs. Meanings of the symbols: A=analyte (tryptophane enantiomers); C=carbonate zone; 1,2=front and rear spacing constituents, respectively. CD=conductivity detector (see Fig. 1); UV=photometric absorbance detector (see Fig. 1).

employed for the ITP sample pretreatment was identical with the carrier anion of the electrolyte system used in the CZE separation stage (Table 1) to preserve a constant composition of the carrier electrolyte during the final CZE separation.

An electropherogram obtained from the ITP-CZE separation of a model mixture containing tryptophan enantiomers (Fig. 3) in the working mode A (see

above) illustrates the CZE resolution of the two when their ratio in the sample was 1:200 and their detectabilities (note that the concentration of L(-)-Tryptophan was 25 nmol/l). From the data obtained in experiments with low concentrations of the enantiomers we estimated that their limits of detection (LODs) were at ~10 nmol/l (~1.5 ng/ml) concentrations for a 220 nm detection wavelength of the

Table 1  
Electrolyte system

ITP		CZE	
Solvent	Water	Solvent	Water
Leading anion	Chloride	Carrier anion	Borate
Concentration (mmol/l)	10	Concentration (mmol/l)	50
Counterion	BTP	Counterion	BTP
Additive	MHEC	Additive	MHEC
Concentration (% w/v)	0.2	Concentration (% w/v)	0.2
pH	9.3	Chiral selector	$\alpha$ -CD
Solvent	Water	Concentration (mmol/l)	80
Terminating anion	Borate	pH	9.0
Concentration (mmol/l)	10		
Counterion	BTP		
PH	9.0		

BTP=bis-tris propane; MHEC=methylhydroxyethylcellulose.

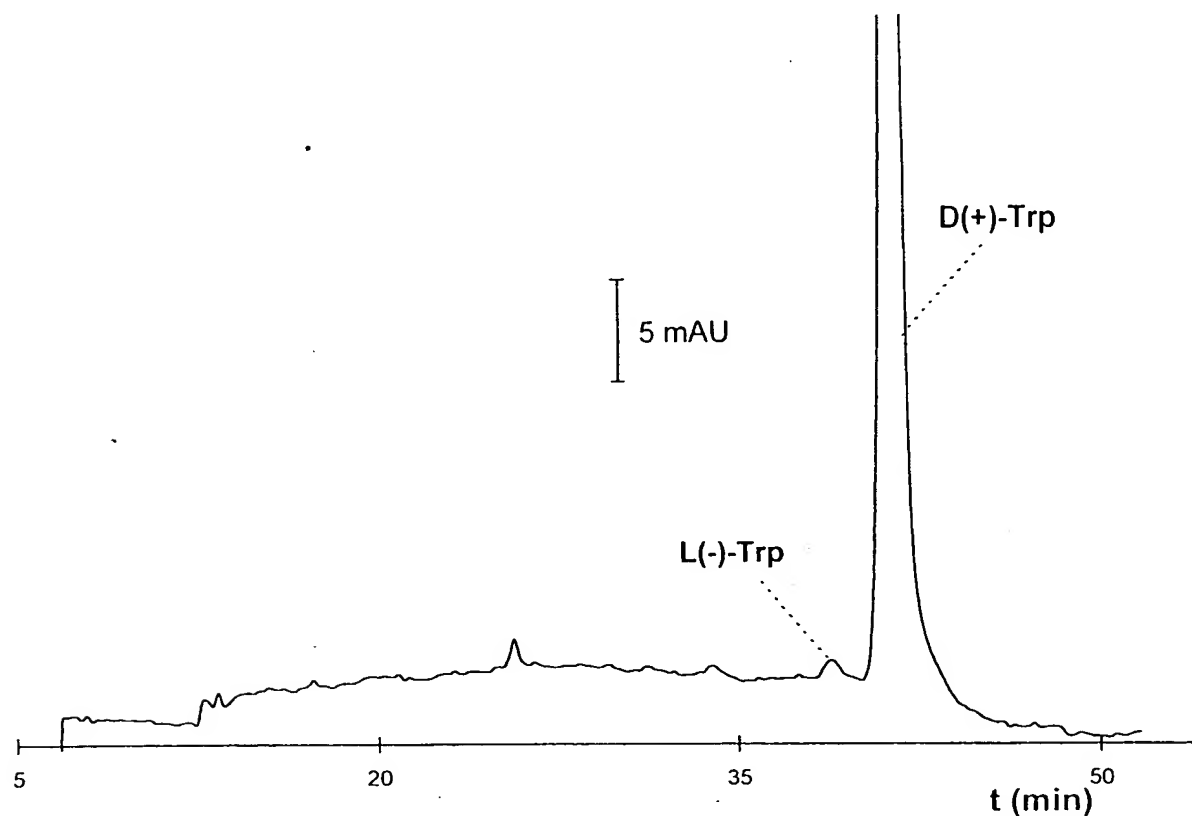


Fig. 3. An electropherogram from the separation of tryptophan enantiomers by the ITP–CZE combination. The injected model sample (30  $\mu$ l) contained L(–)-Trp at a 25 nmol/l concentration and D(+)-Trp at a 5000 nmol/l concentration. The electrolyte system used is described in Table 1. The driving currents were 250 and 70  $\mu$ A in the ITP and CZE stages, respectively. The detector used in the CZE stage was set at a 220 nm detection wavelength.

UV detector employed in the CZE stage and for a 30  $\mu$ l sample volume.

### 3.2. Separation of tryptophan enantiomers present in a 90-component model mixture

A 90-component mixture of UV-light absorbing organic acids (see 2.2.) served in our experiments as a model multicomponent sample matrix. The ITP–CZE separations of the enantiomers present in such a sample matrix were aimed at comparing analytical possibilities of various working modes of the ITP–CZE combination (see Fig. 2) in situations when a multicomponent character of the samples need to be taken into account. Electropherograms in Fig. 4 (a–c) were obtained from these comparative experiments with the model matrix spiked with the tryptophan enantiomers at sub- $\mu$ mol/l concentrations. These electropherograms clearly show that the best results in terms of potential matrix interferences were achieved when the ITP stage was used as an effective sample clean-up technique (Fig. 4 (c)). This is understandable as in this instance only the sample fraction covering a narrow mobility span (defined by the spacing constituents) was transferred into the CZE column after the ITP sample pretreatment. In addition, the electropherograms also indicate a positive role of different separation mechanisms determining the electrophoretic migration of tryptophan in both separation stages [35]. Typical repeatabilities of the migration times and peak areas of the enantiomers show (Table 2) that these scattered within narrow limits also in situations when they were present in the injected model samples at 100 nmol/l concentrations.

### 3.3. Separation of tryptophan enantiomers in urine matrices

Urine besides its multicomponent anionic matrix characterizes also high concentrations of inorganic ions [7]. Therefore, a high sample load capacity of the ITP stage is essential when enantiomers present in urine at low concentrations are to be separated and/or determined by the ITP–CZE combination. The concentration of the leading anion and a large effective volume of the ITP column (ca. 90  $\mu$ l) were logical means used in our experiments to meet such

sample load capacity requirements [37]. For example, under working conditions employed in this work we could load onto the separation system the samples corresponding to 3–6  $\mu$ l volumes of undiluted urine while still achieving a full recovery of the analyte. This was a 300–600 fold increase in comparison to current loads of urine samples in CZE [11].

CZE combined with a transient ITP sample stacking (Fig. 2 (B)) and CZE combined with a post-column ITP sample clean-up (Fig. 2 (C)) were the working modes of the ITP–CZE combination used in the detection and tentative determination of L(–)-tryptophan present in urine. Electropherograms in Figs. 5 and 6 illustrate typical results as obtained in the analysis of a series of urine samples. Here, it is clear that both working modes provided quantifiable L(–)-tryptophan peaks for the urine samples characterized by a less complex anionic matrix (the electropherograms in Fig. 5). On the other hand, from the electropherograms in Fig. 6 we can see that for urine samples of more complex anionic matrices the use of the post-column ITP sample clean-up was essential to make the analyte in the CZE stage detectable. In this particular instance the post-column ITP sample clean-up [35] was effective in removing more than 99% of the detectable sample anionic constituents (as estimated from the total peak areas on both electropherograms). Undoubtedly, such a clean-up effect can be ascribed to a transfer of the sample fraction corresponding to a narrow mobility span into the CZE stage (defined by the spacing constituents as marked in Fig. 6 (b)). Different separation mechanisms responsible for the electrophoretic migration of tryptophan in the ITP and CZE stages can be assumed to minimize the number of comigrating matrix constituents under the peak of L(–)-tryptophan [35]. Repeatabilities of the migration times and peak areas of this enantiomer given in Table 3 show that also for such a complex urine matrix these parameters scattered within limits comparable to those obtained for a more favourable model matrix (see Table 2).

From a general viewpoint the ITP–CZE combination performed in the column-coupling CE separation system can be considered as one of the CE analogies of the column-switching chromatography separation systems [10]. Despite some operational limitations

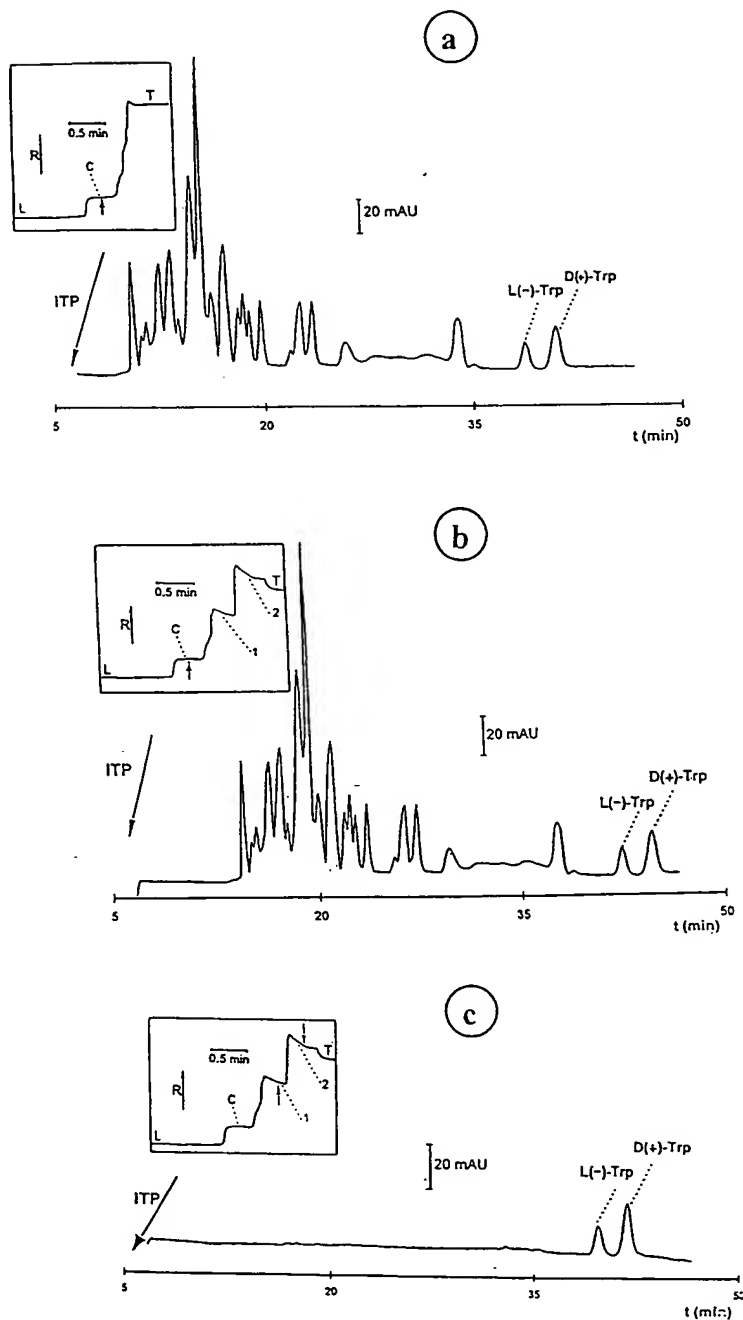


Fig. 4. Electropherograms from the separations of tryptophan enantiomers present in a 90 component model mixture of organic acids using various working modes of the ITP-CZE combination. a,b,c=the electropherograms corresponding to the working modes A,B,C, respectively (see Fig. 2). L(-)-Trp and D(+)-Trp were present in the injected mixture (30  $\mu$ l) at 0.5  $\mu$ mol/l and 0.8  $\mu$ mol/l concentrations, respectively. The concentrations of organic acids in the model mixture were in the range of 1–5  $\mu$ mol/l. Arrows on the isotachopherograms mark the sample fractions taken for final CZE separations. Glycine (1) and  $\beta$ -alanine (2) added to the sample at 1 mmol/l concentrations served as discrete spacing constituents in the ITP stage of the separation. The electrolyte system used is described in Table 1. The driving currents were 250 and 70  $\mu$ A in the ITP and CZE stages, respectively. The detector used in the CZE stage was set at a 220 nm detection wavelength.



Table 2  
Repeatabilities of the migration times and peak areas of tryptophan enantiomers in the ITP–CZE runs with a model mixture

Run No.	D,L-tryptophan <sup>a</sup>				
	$t_L$ (min)	$A_L$ (mAU s)	$t_D$ (min)	$A_D$ (mAU s)	$R_s$
1	38.61	316 087	40.93	330 385	1.39
2	38.50	336 960	41.00	339 530	1.40
3	38.34	330 275	40.69	340 935	1.40
4	38.36	340 426	40.39	332 980	1.38
5	38.37	332 038	40.40	329 420	1.40
Mean	38.44	331 157	40.68	334 650	1.39
R.S.D.(%)	0.27	2.52	0.63	1.41	0.57

<sup>a</sup> The concentrations of enantiomers in the injected sample were 100 nmol/l.

$t_L$ ,  $t_D$ =migration times of L(–) and D(+) enantiomers in the CZE stage, respectively.

$A_L$ ,  $A_D$ =peak areas of L(–) and D(+) enantiomers, respectively.

$R_s$ =resolution.

such chromatography separation systems provided with achiral and chiral columns may be used, e.g., in the liquid chromatography separations of enantiomers of chiral compounds present in biological samples (see, e.g., ref. [38] for a review of this topic). As could be expected, our experiments carried out with both the model and urine samples showed that the ITP–CZE combination in the column-coupling separation system was much simpler to operate than its chromatography counterparts (1–2 min lasting replenishments of the electrolyte solutions in the ITP and CZE columns between the runs were the only operations needed to start a run with a new sample). This appears to be a significant practical advantage of the ITP–CZE combination over liquid chromatography with coupled achiral and chiral columns.

#### 4. Conclusions

Our results clearly demonstrate capabilities of the ITP sample pretreatment in CZE and, in fact, general potentialities of the ITP–CZE combination in the column-coupling separation system to separate and/or determine enantiomers present in biological fluids. These potentialities are closely associated with the following features of the combination:

1. The ITP stage of the ITP–CZE combination significantly enhances the sample load capacity of the CE separation system and, consequently,

considerably improves concentration LODs attainable for enantiomers in CZE.

2. A concentrating capability of the ITP separation process significantly reduce the volume in which the analyte (a mixture of enantiomers) is present so it can be transferred into the CZE stage with a minimum injection dispersion.
3. A post-column ITP sample clean-up is provided by the column-switching operation of the separation system. This capability combined with appropriately chosen spacing constituents can considerably reduce the number of sample constituents transferred onto the CZE column.
4. Different separation mechanisms can be applied in both stages of the ITP–CZE combination. This allows to combine a separation mechanism favouring the resolutions of the enantiomers in the CZE stage with the one suitable for their isolations from the sample matrix in the ITP stage. These two-dimensional features of the combination minimize risks that the peaks of the enantiomers are overlapped with the peaks of the sample matrix constituents in the CZE stage.

Urine samples contain proteins at low concentrations [39] and, therefore, our results cannot characterize capabilities of the ITP–CZE combination in the analysis of enantiomers present in protein rich matrices. An experimental evaluation of the combination in this respect is needed. However, a recent work by Krivánková et al. with serum samples [30] indicates that the presence of proteins in the samples

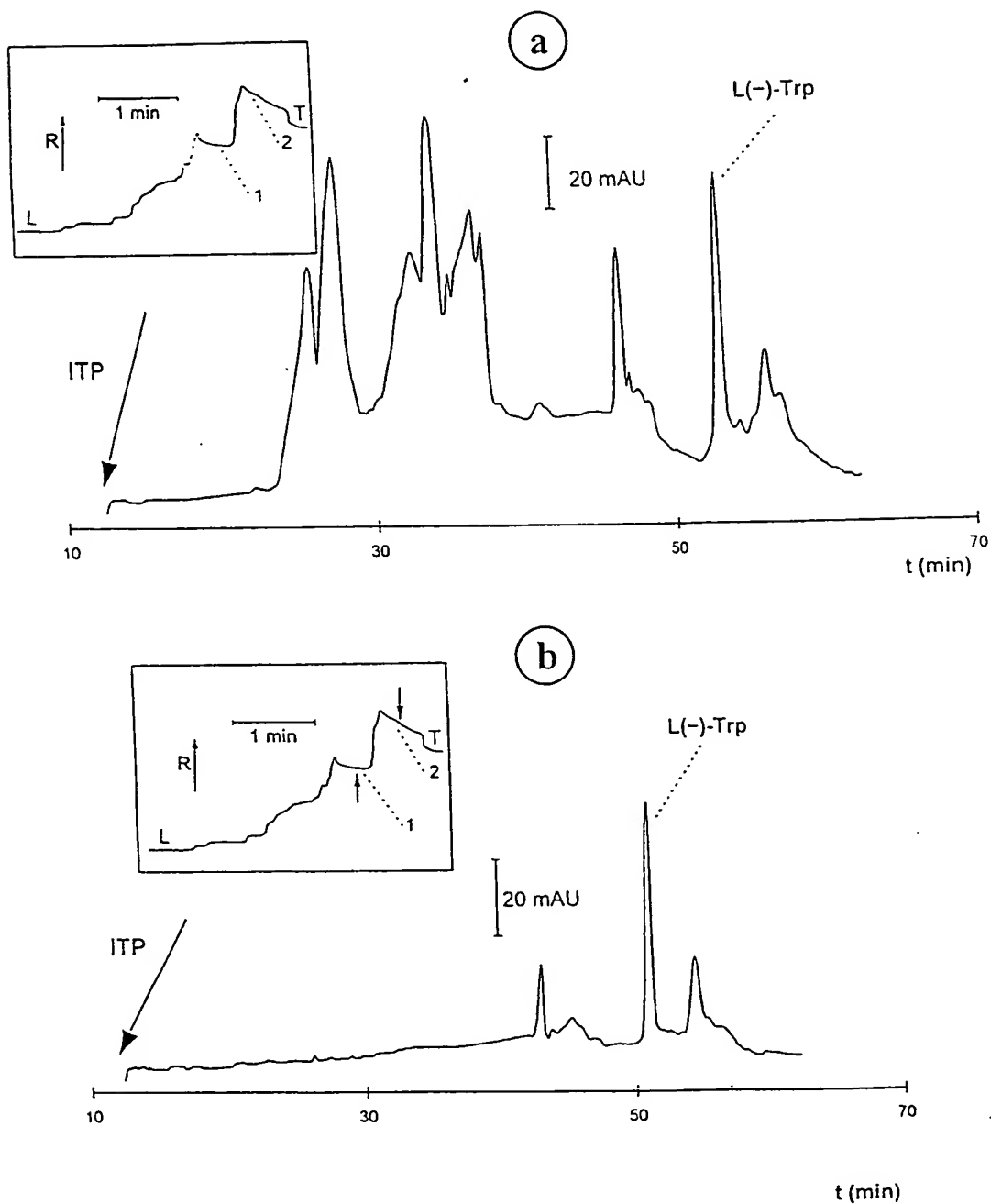


Fig. 5. Electropherograms from the separations of tryptophan enantiomers in a urine sample using various working modes of the ITP–CZE combination (for a schematic description of the working modes see Fig. 2). a=working mode B; b=working mode C. Arrows on the isotachopherograms mark the sample fractions taken for final CZE separations. Glycine (1) and  $\beta$ -alanine (2) added to urine at 1 mmol/l concentrations served as discrete spacing constituents in the ITP stage of the separation. The urine sample (a mid-stream fraction) was diluted 1:5 (v/v) with demineralized water immediately after the collection. It was analyzed without any further sample preparation. L(-)-Trp was estimated to be present in the sample at a 3.8  $\mu\text{mol/l}$  concentration. The electrolyte system used is described in Table 1. The driving currents were 250 and 70  $\mu\text{A}$  in the ITP and CZE stages, respectively. The detector used in the CZE stage was set at a 220 nm detection wavelength.

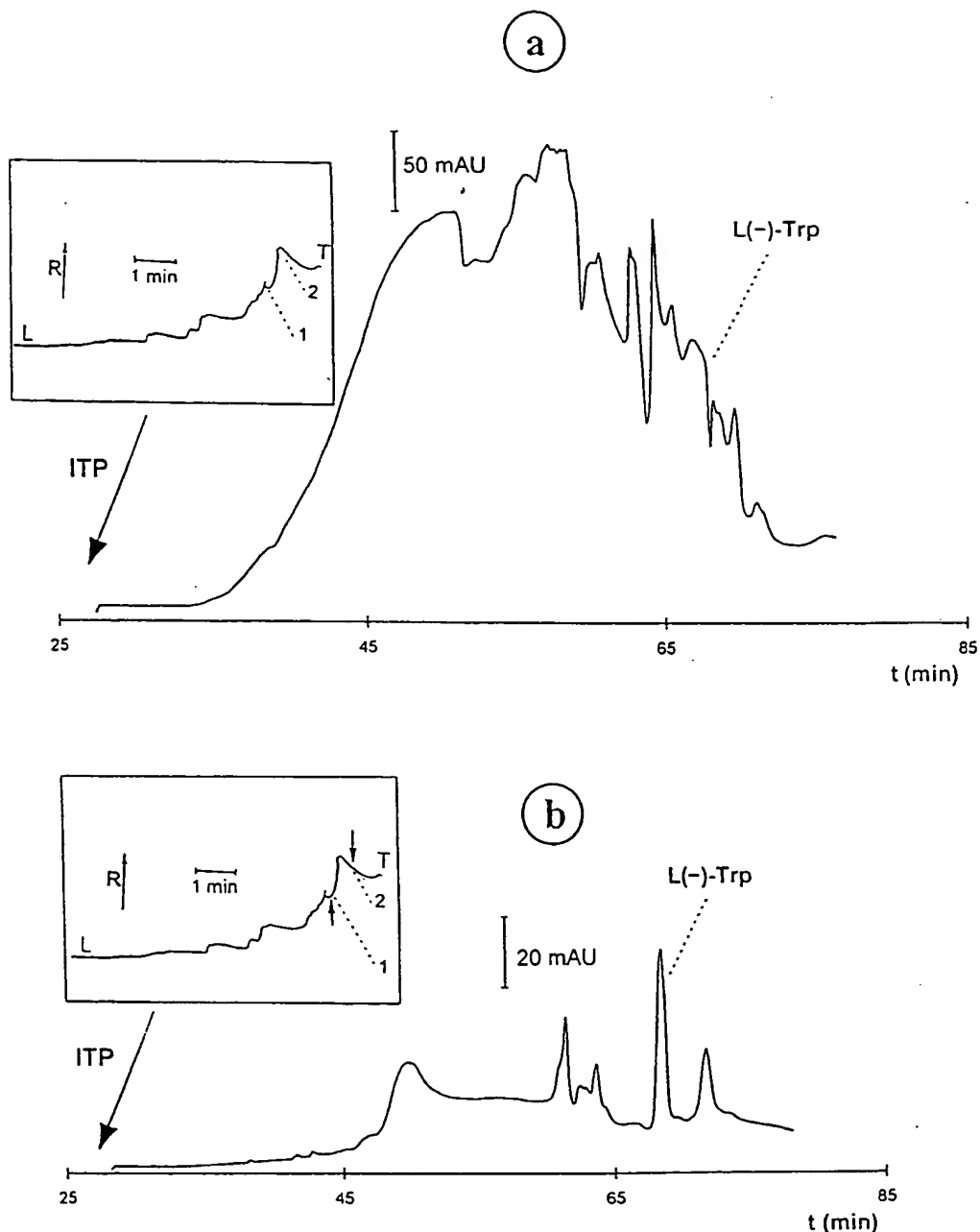


Fig. 6. Electropherograms from the separations of tryptophan enantiomers present in a urine sample illustrating importance of the post-column ITP sample clean-up in the ITP-CZE combination. a=working mode B; b=working mode C (for a schematic description of the working modes see Fig. 2). Arrows on the isotachopherograms mark the sample fractions taken for final CZE separations. Glycine (1) and  $\beta$ -alanine (2) added to urine at 1 mmol/l concentrations served as discrete spacing constituents in the ITP stage of the separation. The urine sample (a mid-stream fraction) was diluted 1:5 (v/v) with water immediately after the collection. It was analyzed without any further sample preparation. L(-)-Trp was estimated to be present in the sample at a 4  $\mu$ mol/l concentration. The electrolyte system used is described in Table 1. The driving currents were 250 and 70  $\mu$ A in the ITP and CZE stages, respectively. The detector used in the CZE stage was set at a 220 nm detection wavelength.

Table 3

Repeatabilities of the migration times and peak areas of L(–)-tryptophan in the ITP–CZE runs with a urine sample \*

Run No.	$t_L$ (min)	A (mAU s)
1	41.13	810 088
2	41.10	813 678
3	41.69	825 986
4	41.18	835 690
5	42.10	805 480
Mean	41.44	818 184
R.S.D. (%)	0.95	1.36

\* The working mode C (Fig. 2) was used with the injection of a urine sample to which glycine and  $\beta$ -alanine (the spacing constituents) were added; the analyte fraction was transferred into the CZE column with a 70  $\mu$ A driving current and included besides the analyte zone also neighbouring parts of the zones of front (glycine) and rear ( $\beta$ -alanine) spacers (the sizes of the transferred parts of the zones of spacers corresponded to 2.38 mC in the charge units).

$t_L$  = migration times of L(–) enantiomer in the CZE stage.

A<sub>L</sub> = peak areas of L(–) enantiomers.

should not act restrictively in its use to the separations of enantiomers.

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